# CHERYL HEATHER AGRIS

P.O. Box 806
Pelham, New York 10803
(914) 712-0093 (telephone)
(914) 712-0094/5 (telefax)
c.agris.patlaw@pobox.com


## DEGREE PROGRAMS:

1992: J.D., Brooklyn Law School
Top 15%

1986: Ph.D., The Johns Hopkins University, Baltimore, Maryland
School of Hygiene and Public Health
Department of Biochemistry, Division of Biophysics
Thesis: "Effects of Oligonucleoside Methylphosphonates on Vesicular Stomatitis Virus Protein Synthesis and Infection"

1979: Bachelor of Arts, Chemistry, Goucher College,
Towson, Maryland
Cumulative grade point average: 3.57/4.00

## CONTINUING EDUCATION:

2000: NASD Arbitrator Training Program; Writing and Using Intellectual Property Opinions (Association of the Bar of the City of New York); International Intellectual Property Symposium, Brooklyn Law School; Preparing Legal Opinions
1999: Intellectual Property Due Diligence in Business Transactions (Association of the Bar of the City of New York)

1998: New York, New Jersey, Connecticut, and Pennsylvania Joint Seminar on Developments in Patent Law

1996: The Basics of Licensing and Licensing Law (The Licensing Journal)

1995: Patent Aspects of GATT (ABA, Section of Intellectual Property Law)
Advanced PCT Practice (Patent Resources Group)

1994: European Patent Office Practice (Patent Resources Group)

1993: Technology Licensing and Litigation (Practicing Law Institute)

## ADMISSIONS:

New York and New Jersey State Bars
Registered to practice before the U.S. Patent and Trademark Office

## LEGAL EXPERIENCE:

1998-present: Solo practitioner
Preparation and prosecution of U.S. patent applications in the biotechnology, pharmaceutical and chemical fields; overseeing foreign prosecution of patent applications; patentability and validity studies; infringement analysis; freedom of operation studies; preparation of licensing, consulting and confidentiality agreements; peer review of patent prosecution by third parties; arbitrator

1992-1998: Patent Attorney, Novo Nordisk of North America, N.Y., N.Y.
Preparation and prosecution of U.S. patent applications in the biotechnology and chemical fields; prosecution of foreign applications in the United States in the biotechnology, chemical, and pharmaceutical fields; supervising foreign filings; supervising patent liaison in California subsidiary; patentability and validity studies; infringement analysis; and preparation of licensing, consulting, confidentiality, and research agreements

1988-1992: Pennie & Edmonds, Law Clerk, Biotechnology Group
Preparation and prosecution of U.S. patent applications in the biotechnology, chemical and pharmaceutical fields and interactions with foreign associates regarding foreign prosecution; assisted in patentability studies, validity studies, and infringement analyses and assisted in the preparation of consulting, confidentiality and licensing agreements

## PRE-LEGAL EXPERIENCE:

1986 -1988: Research Fellow, Sloan Kettering Institute
Investigated the mechanism of the block in splicing of influenza viral NS1 mRNA to NS2 mRNA in vitro using molecular biological and biochemical approaches

1979-1986: Predoctoral Fellow, Johns Hopkins University
Formulated methods for synthesizing the antisense nonionic oligonucleotide analogs, oligonucleoside methylphosphonates; studied the effects of oligonucleoside methylphosphonate sequences on the synthesis of VSV (vesicular stomatitis virus) proteins in cell culture and in vitro

1979: Undergraduate Research Associate, Argonne National Laboratory.
Analyzed bile acids isolated from the bile, urine, or serum from children with cholestatic liver disease using gas chromatography and gas chromatography/mass spectroscopy

## HONORS, AWARDS, AND FELLOWSHIPS:

1988-1992: Richardson Scholar, Brooklyn Law School

1988-1990: Dean's List, Brooklyn Law School

1986-1988: American Cancer Society Postdoctoral Fellow,
Sloan Kettering Institute

1984: Student Research Award, Delta Omega Honorary
Public Health Society

1979-1986: Predoctoral Training Grants: Predoctoral trainee, NIH (1979-1982);
Albert Szent-Gyorgyi Foundation (1982-1986)

May 1979: Graduated with General Honors and Honors in Chemistry from Goucher College
Louise Kelly Award in Chemistry, Goucher College

1979: Undergraduate Research Program (January-May) and Summer Graduate Student Program (June-August) at Argonne National Laboratory, Argonne, Illinois

## FACULTY APPOINTMENTS

Angel Financing:Navigating the Legal & Business Issues, Citybar Center for CLE, The Association of the Bar of the City of New York, November 28, 2000

Organizer and instructor at National Association of Patent Practitioner's 2000 Short Course on Nuts and Bolts of Patent Prosecution, July 2000

Instructor, Sixth, Eighth, Ninth and Tenth Annual Patent Prosecution Workshops: Advanced Claim Drafting and Amendment Writing (1996, 1998, 1999, 2000)

Law Seminars International: Biotechnology Key Legal & Business Issues, November 18-19, 1999, Seattle Washington

## ADDITIONAL SKILLS

Computer literate, LEXIS, WESTLAW, DIALOG, Internet User

## MEMBERSHIPS:

National Association of Patent Practitioners: Member, Board of Directors and Chairperson of Education Committee
American Intellectual Property Law Association
Eastern New York Intellectual Property Law Association
American Bar Association, Intellectual Property Section
Association of the Bar of the City of New York
Association for Women in Science
Association of University Transfer Managers
Westchester Women's Bar Association
International Intellectual Property Society

## ORAL PRESENTATIONS

"Alternative Career Opportunities in Intellectual Property Property Law", New York Biotechnology Association, Women in Bioseciences Section Meeting, June 2000

"Why Deposit Biological Materials?" New York, New Jersey, Connecticut, and Pennsylvania Joint Seminar on Developments in Patent Law, April 2000

"Inventorship", National Association of Patent Practitioners meeting, July 1999
Panel chair, "Interactions Between In-House and Law Firm Patent Counsel to Develop Intellectual Property Strategy", BIO '98, June 1998

"What to Claim in Biotechnology Patent Applications", National Association of Patent Practitioners meeting, October 1997

"*In re Deuel,* Obviousness Standard for Biotechnology", BIO '96, June 1996

## PUBLICATIONS:

### Patents:

Have participated in the preparation and/or prosecution of over 150 patents. Representative patents are listed below:

U.S. Patent No. 6,060,305, "Non-toxic, non-toxigenic, non-pathogenic Fusarium expression system"

U.S. Patent No. 5,919,697, "Color Clarification Methods"

U.S. Patent No. 5,843,753, "Metalloprotease having increased activity"

U.S. Patent No. 5,770,371, "Modification of cryptic splice sites in heterologous genes expressed in fungi"

U.S. Patent No. 5,726,202, "Benign prostatic hypertrophy"

U.S. Patent No. 5,707,798, "Identification of ligands by selective amplification of cells transfected with receptors"

U.S. Patent No. 5,602,032, "Bacillus thuringiensis mutants which produce high yields of crystal delta-endotoxin"

U.S. Patent No. 5,580,560, "Modified factor VII/VIIa"

U.S. Patent No. 5,525,193, "Use of monocomponent cellulase for removing inks, coatings, and toners from printed paper"

U.S. Patent No. 5,354,760, "Crystalline Tiagabine monohydrate, its preparation and use"

**Legal-related Publications:**

Agris, C.H. (2000) "Biotechnology Applications: Depositing Biological Materials" Intellectual Property Today 7:12-13

Agris, C.H. (1999) "Patenting Plants: What to Claim", Nature BioTechnology 17:717-718

Agris, C.H. (1999) "Intellectual property protection for plants", Nature BioTechnology 17:197-198

Agris, C.H. (1998) "Patenting Protein Sequences", Nature BioTechnology 16:1075

Agris, C.H. (1998) "Patenting DNA Sequences", Nature BioTechnology 16:877

Agris, C.H. (1998) "International Patent Filing", Nature BioTechnology 16:479-480

Agris, C.H. (1996) "Prior Art Considerations When Patenting DNA Sequences", Nature Biotechnology 14:1309-1310

**Scientific Publications and Abstracts:**

Agris, C.H.; Nemeroff, M. E. and Krug, R. M. (1989) A block in mammalian splicing occurring after formation of large complexes containing U1, U2, U4, U5 and U6 small nuclear ribonucleoproteins, Mol Cell. Biol. 9:259-262

Ts'o, P.O.P; Miller, P S; Aurelian, L.; Murakami, A.; Agris, C.; Blake, K.R.; Lin, S-B ; Lee, B.L. and Smith, C.C. (1987), An approach to chemotherapy based on base sequence information and nucleic acid chemistry. Matagen (masking tape for gene expression), Ann. NY Acad. Sci. 507:220-241

Agris, C.H.; Plotch, S.J. and Krug, R.M. (1986) "In vitro splicing of influenza viral NS1 mRNA and NS1-β-globin chimeras: possible mechanisms for the control of viral mRNA splicing, Memorial Sloan-Kettering Cancer Center Research Colloquium, Abstract #11.

Miller, P S; Agris, C.H.; Aurelian, L.; Blake, K.R.; Glave, S.A.; Lin, S-B; Murakami, A.; Reddy, M.P.; Smith, C.C.; Spitz, S.A.and Ts'o, P.O.P (1987), Matagen: (masking tape for gene expression): A family of sequence specific oligonucleoside methylphosphonates, Working group on: Molecular mechanisms of carcinogenic and antitumor activity, Vatican City, Italy, October 21-25, 1986, Pontif. Acad. Sci. Scr. Varia 70:169-204

Miller, P.S.; Agris, C.H.; Blake, K.R.; Lee, B.L.; Lin, S-B; Spitz, S.A.and Ts'o, P.O.P. (1986), Control of Nucleic-acid expression by oligonucleoside methylphosphonates J. Cell Biol. 103: PA437

Agris, C.; Blake, K. R.; Miller, P. S.; Reddy, M.P. and Ts'o, P.O.P (1986) Effects of oligodeoxyribonucleoside methylphosphonates on vesicular stomatitis virus protein synthesis and infection, Biochemistry 25:6268-6275

Miller, P.S.; Reddy, M.P.; Murakami, A.; Blake, K.R.; Lin, S.B. and Agris, C.H. (1986) Solid-phase syntheses of oligodeoxyribonucleoside methylphosphonates Biochemistry 25:5092-5097

Miller, P.S.; Agris, C.H.; Aurelian, L.; Blake, K.R.; Lin, S.B.; Murakami, A.; Reddy, M.P.; Spitz, S.A.and Ts'o, O.P. (1985) Control of ribonucleic acid function by oligonucleoside methylphosphonates, Biochimie 67: 769-776

Miller, P.S.; Agris, C.H.; Aurelian, L.; Blake, K.R.; Lin, S.B.; Murakami, A; Reddy, M.P.; Smith, C.and Ts'o, P.O.P. (1985) Control of gene expression by oligonucleoside methylphosphonates. In Interrelationship Among Aging, Cancer and Differentiation, B. Pullman and J. Jortner, eds. (D. Reidel Publishing Co., Boston), pp. 207-209

Reddy, M.P.; Murakami, A.; Agris, C.H.; Glave, S.A.; Miller, P.S. and Ts'o (1984) Solid phase synthesis of oligonucleoside methylphosphonates, Biochemistry 23:3371, 1984

Miller, P.S., Agris, C.H., Aurelian, L., Blake, K., Kelly, T., Murakami, A., Reddy, M.P., Spitz, S., Ts'o, P. and Wides, R. (1984) Inhibition of viral protein synthesis and infection by oligonucleoside methylphosphonates (OMNP), Fed. Proc. 43:1727

Miller, P.S.; Agris, C.H.; Murakami, P.M.; Reddy, P.M.; Spitz, S.A. and Ts'o, P.O.P. (1983) Preparation of oligodeoxyribonucleoside methylphosphonates on a polystyrene support, Nucleic Acids Res. 11: 6225-6242

Miller, P.S.; Agris, C.H.; Blandin, M. Murakami, A., Reddy, P.M. Spitz, S.A. and Ts'o, P.O.P. (1983) Use of methylphosphonic dichloride for the synthesis of oligonucleoside methylphosphonates. Nucleic Acids Res. 11:5189-5204

Miller, P.S., Agris, C.H., Blake, K.R., Murakami, A., Spitz, S.A., Reddy, P.M. and Ts'o, P.O.P. (1983) Nonionic oligonucleotide analogs as new tools for studies on the structure and function of nucleic acids inside living cells. In Nucleic Acids: The Vectors of Life, B. Pullman and J. Jortner, ed. (D. Reidel Publishing Co., Boston), pp. 521-535

# Effects of Chemically Synthesized Oligodeoxyribonucleoside Methylphosphonates on Vesicular Stomatitis Virus Protein Synthesis and Infection

by

Cheryl Heather Agris

A dissertation submitted to the Johns Hopkins University in conformity with the requirements for the degree of Doctor of Philosophy

Baltimore, MD

1986

# II. Background

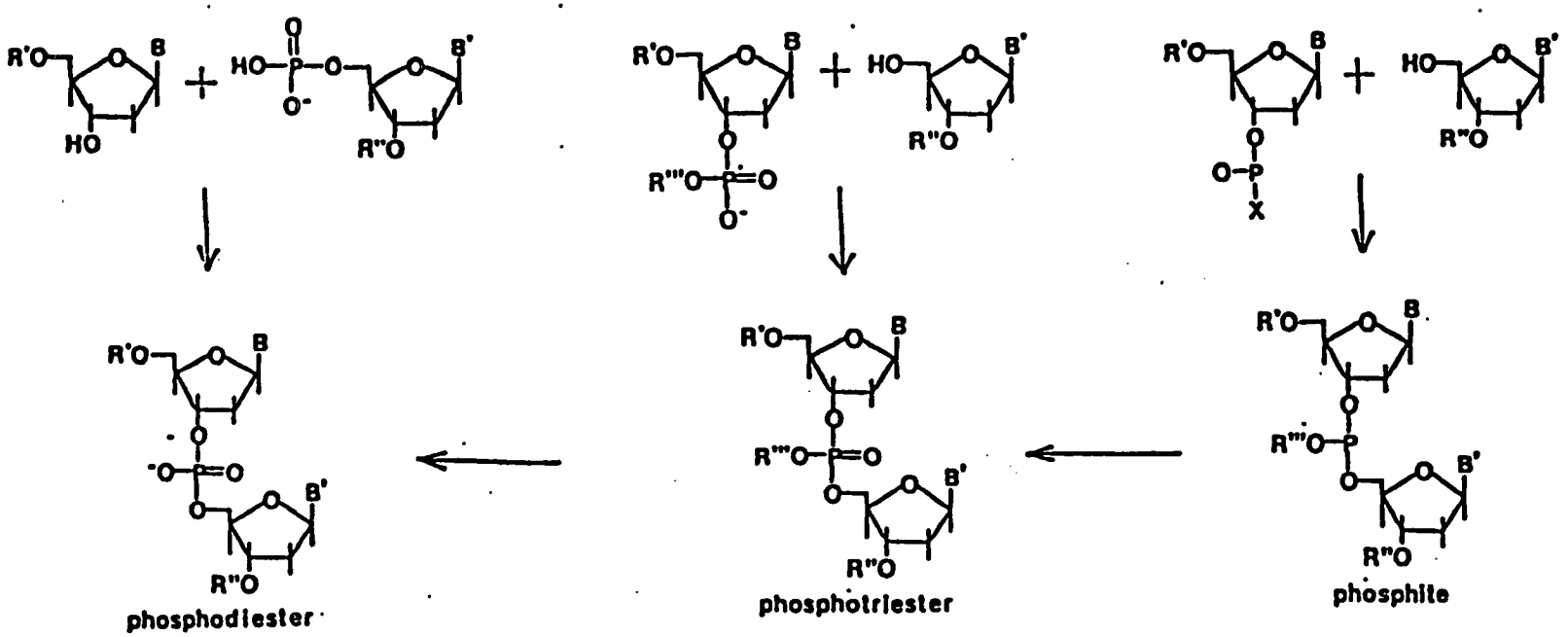
## A. Synthesis of oligodeoxyribonucleotides and oligodeoxyribonucleoside methylphosphonates

### 1. Synthesis of oligodeoxyribonucleotides

The chemical synthesis of oligodeoxyribonucleotides involves the formation of phosphodiester internucleotide linkages. It has been accomplished by the activation of phosphomonoesters (phosphodiester method) (Jacob and Khorana, 1964), phosphodiesters (phosphotriester method) (Letsinger and Ogilvie, 1969), and phosphites (phosphite method) (Letsinger and Lunsford, 1976) (see figure 1). The reaction between a nucleotide and nucleoside to form an internucleotide linkage is a condensation reaction. All functional groups except for those involved in the condensation reaction must be blocked with protecting groups to prevent the formation of side products. These groups are removed at the conclusion of the condensation reaction.

The 5'-hydroxyl group is commonly blocked by one of a family of ether groups which are increasingly labile to acid in the order trityl < monomethoxytrityl < dimethoxytrityl (Schaller et al., 1963). The structures of these compounds are shown in figure 2. Since the amino group of the bases are reactive, they are protected with a benzoyl group in the case of adenine and cytidine (Schaller et al., 1963) and with an isobutyryl moiety in the case of guanine (Agarwal et al.,

Figure 1. Phosphodiester, phosphotriester, and phosphite methods for the preparation of dideoxyribonucleotides.

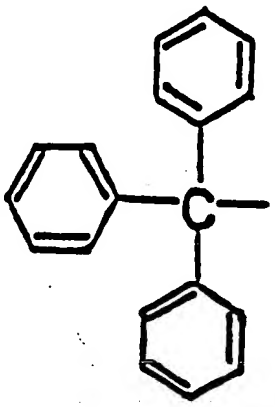



R' = trityl, monomethoxytrityl, dimethoxytrityl
(see fig. 2 for structures)

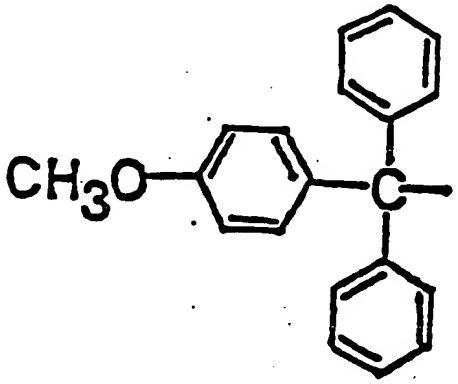
R'' = $-OOCH_3$, $-Si(CH_3)_2-C(CH_3)_3$, $-OC_6H_5$

R''' = $-CH_2CH_2CN$, $-pClC_6H_4$, $-oClC_6H_4$, $-CH_3$

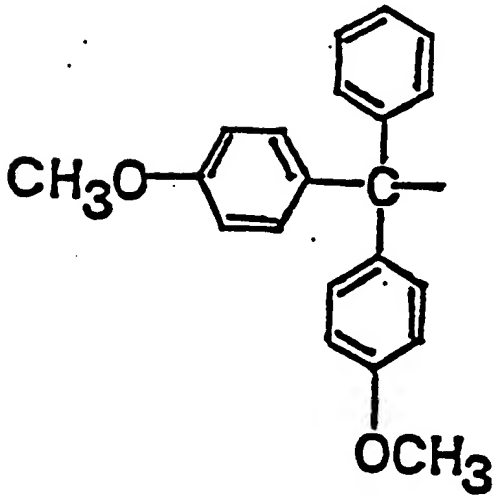
X = $-Cl$, $-N(CH_3)_2$



trityl




monomethoxytrityl




dimethoxytrityl

Figure 2. 5'-protecting groups

1972) (see figure 3). Both the isobutyryl and benzoyl groups ar base labile. A number of adducts have been used to block the 3'-hydroxyl group, e.g. acetyl (OAc) (Agarwal et al., 1972) or t-butyldimethylsilyl (OTBS) adducts (Ogilvie, 1973). Since the acetyl group is base labile, the base protecting groups may be partially removed as well. This may not be desirable if a polynucleotide is being synthesized. The t-butyldimethylsilyl group can be removed by fluoride treatment.

Three groups are most commonly used to protect the phosphate linkage when the phosphotriester or phosphite approaches are used. No protecting group was used in the phosphodiester method. The β-cyanoethyl group has been used in the phosphotriester (Letsinger et al., 1969) and phosphite methods (Sinha et al., 1984). This group is removed by β-elimination using mild alkaline treatments. The o- and p-chlorophenyl derivatives were developed in connection with the phosphotriester method (Katagiri et al., 1975). These groups can be selectively removed by tetramethylguanidium pyridine aldoximate (Reese et al., 1978), a mild nucleophile. The methyl group has been developed for use in the phosphite method and is removed by thiophenol followed by treatment with concentrated ammonium hydroxide (Matteucci and Caruthers, 1981). The o- and p-chlorophenyl and methyl protecting groups are not interchangeable between synthetic methods since it is virtually impossible to make chlorophenyl phosphitylating

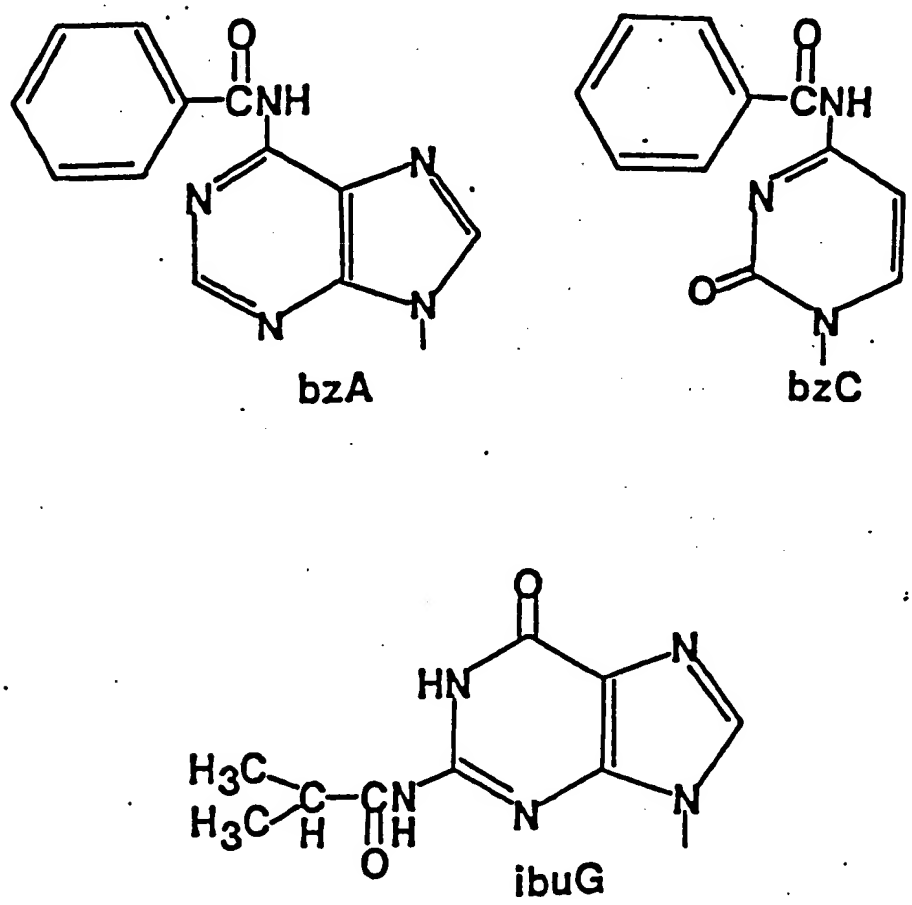



Figure 3. Base protecting groups

agents and methyl substituted phosphorylating agents are not very reactive (Gait, 1984).

As shown in figure 1, the phosphodiester approach involves direct condensation of oligonucleotides bearing a 5'-phosphomonoester group with oligonucleotides possessing a free terminal 3'-hydroxyl group. Originally, the phosphomonoester group was activated with dicyclohexylcarbodiimide (Jacob and Khorana, 1964). Faster activation was found to be achieved with such arenesulfonyl chlorides as mesitylenesulfonyl chloride or triisopropylbenzenesulfonyl chloride (Lohrmann and Khorana, 1965). The major drawback to this approach is that the phosphodiesters in the internucleotide linkages are also activated by these condensing agents resulting in the formation of side products with 3'-3' internucleotide linkages.

In the phosphotriester method, all hydroxyl groups are protected, thereby eliminating the 3'-3' side product (Katagiri et al., 1975). However sulfonated side products were still present when arenesulfonyl chlorides were used as condensing agents. In an effort to circumvent this problem and to decrease the reaction times, more active derivatives were introduced. These included arenesulfonyl triazolides (Katagiri et al., 1975), arenesulfonyl tetrazolides (Stawinski et al., 1977), and later mesitylenesulfonyl-3-nitrotriazole (MSNT) (Reese t al., 1978).

The third approach or phosphite method involves reacting

a protected nucleoside with a bifunctional phosphitylating agent such as methoxydichlorophosphine (Letsinger and Lunsford, 1976). The resulting nucleoside 3'-phosphomonochloridite is then reacted with a second protected nucleoside resulting in the formation of a dinucleoside phosphite. This product is converted to a phosphate by oxidation with iodine. Condensation reactions in which the phosphite method is used are completed faster than those using phosphotriester chemistry. The main disadvantage to this method is that due to its instability, the preparation of the phosphomonochloridate must be carried out at -78°C. There is also considerable formation of 3'-3' dimer. In an effort to overcome these disadvantages, phosphoramidite derivatives of the nucleoside phosphomonochloridite were prepared (Beaucage and Caruthers, 1981). These intermediates have been found to be stable and can be activated in the presence of tetrazole to form the internucleotide bond.

Oligodeoxyribonucleotides may be synthesized in solution or on a solid polymer support. The solution method has the following advantages (Ohtsuka et al., 1982): 1) Relatively large quantities of materials (10-100 mg) can be synthesized. 2) The ratio of 3' to 5' components used in the condensation reactions are usually in the range of 1.5 to 2.0 equivalents. Thus, large excesses of reagents are not required. The major disadvantage of the solution method is that protected oligonucleotides must be purified after each condensation

step. This purification is usually carried out by silica gel column chromatography (Miller et al., 1980).

Oligodeoxyribonucleotides have also been synthesized on a solid polymer support using both the phosphotriester (Ito et. al., 1982) and phosphite approaches (Matteucci and Caruthers, 1981). In this method, the growing oligomer chain is attached to a solid matrix via a linker arm. The growing oligonucleotide chain is therefore purified after each condensation step by a simple filtration and washing procedure. Examples of solid support materials used in oligonucleotide synthesis include polydimethylacrylamide (Gait et al., 1980), polyacrylomorpholide (Miyoshi et al., 1980), silica gel (Matteucci and Caruthers, 1981), polystyrene (Ito et al., 1982), and controlled pore glass (Sproat et al., 1983). Although the method is quite rapid, it appears that rather large excesses (5-20 fold) of incoming nucleotides are required for reactions to go in high yield. It should also be noted that the scale of the synthesis can be reduced since the intermediates need not be isolated after each condensation step. Recently, the operations involved in solid support synthesis have been automated by a number of companies, resulting in the marketing of synthesis machines.

After synthesis of deoxyribonucleotides in solution or on a solid support, all protecting groups are removed. The oligomer is then commonly purified by preparative High Pressure Liquid Chromatography (HPLC) on an ion exchange or

reversed phase column (Miller et al., 1980). Recently, however, a new method has been devised for purifying oligomers by gel electrophoresis (Lo et al., 1984). The final oligomer can then be characterized by HPLC on a reversed phase column and by sequencing using the Maxam-Gilbert procedure (Maxam and Gilbert, 1980).

2. Synthesis of oligodeoxyribonucleoside methylphosphonates

Oligodeoxyribonucleoside methylphosphonates have been synthesized by a number of groups both in solution and on a solid polymer support. Initially, Miller et al. (1979) used mesitylenesulfonyl tetrazolide as a condensing agent in the reaction between a 5'-protected nucleoside 3'-methylphosphonate and a 3'-O-acetylated nucleoside. The 5'-protected nucleoside 3'-methylphosphonate is prepared by esterification of protected nucleosides with methylphosphonic acid in the presence of dicyclohexylcarbodiimide. This procedure is analogous to that used for the preparation of oligonucleoside phosphotriesters (Letsinger and Ogilvie, 1969). Yields were approximately 40-55%.

Agarwal and Riftina (1979) reported the use of methylphosphonic dichloride in combination with benzenesulfonyl tetrazolide as a phosphonylating and condensing agent. In this approach, methylphosphonic dichloride was reacted with 5'-protected nucleoside to prepare 5'-protected nucleoside methylphosphonic chlorides. The intermediate is further activated by benzenesulfonyl

tetrazolide, which is added in the presence of the second nucleoside. They found that when methylphosphonic dichloride was used alone, low yields (12%) were obtained and long reaction times were required.

Our laboratory though has found that with modified reaction conditions that methylphosphonic dichloride can act as a bifunctional phosphonylating/condensing agent to prepare nucleoside 3'-methylphosphonic chlorides and protected di- and trinucleoside methylphosphonates (Miller et al., 1983a). This procedure represented a considerable improvement in time and yield obtained in the preparation of reaction intermediates and di- and trinucleoside methylphosphonates over the method previously used (Miller et al., 1979).

Our laboratory has also synthesized oligonucleoside methylphosphonates on silica gel and 1% divinylbenzene crosslinked polystyrene solid supports. 5'-protected 3'-methylphosphonic chlorides were used as synthetic intermediates when preparing these analogs on a silica gel support (Miller et al., 1983a). Although yields were approximately 70% per step when oligothymidylates were prepared on a silica gel support using this procedure, reactions were less efficient for other nucleosides especially 5'-protected G. Oligomers up to 12 nucleoside units have been prepared in which the triethylammonium salt of 5'-protected nucleoside 3'-methylphosphonic acid is reacted with a nucleoside or oligonucleoside methylphosphonate attached to a

1%-divinylbenzene crosslinked polystyrene solid support with MSNT as the condensing agent (Miller et al., 1983b). Yields have averaged 83% per step. The use of MSNT however can potentially lead to formation of side products resulting from sulfonylation of the nucleoside or 5'-hydroxyl groups. These side products lower the overall yield and complicate purification of the oligomer. In an effort to avoid side reactions, we have recently tested the effectiveness of 5'-protected 3'-methylphosphonic imidazolides as reaction intermediates (Miller et al., 1985b). These reagents contain a leaving group which can be activated just prior to the condensation reaction. This method will be discussed in further detail in later sections of this thesis.

Oligodeoxyribonucleoside methylphosphonates have also been synthesized in solution and on solid supports using methods analogous to the phosphite procedure. One approach involves the use of dichloromethylphosphine as a bifunctional phosphinylating and condensing agent (Engels and Jager, 1982). In this method, 5'-protected 3'-methylphosphine chlorides were used as reactive intermediates in the preparation of oligodeoxyribonucleoside methylphosphonates both in solution (Engels and Jager, 1982) and on a controlled pore glass solid support (Sinha et al., 1983). Dideoxynucleoside methylphosphonates have also been synthesized in solution using nucleoside methylphosphonamidites as reaction intermediates (Jager and Engels, 1984). These compounds have